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Genotyping of immune-related genetic variants identifies *TYK2* as a novel associated locus for idiopathic inflammatory myopathies

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Idiopathic inflammatory myopathies (IIMs) may present as a primary autoimmune disorder, or overlap with other autoimmune/connective tissue diseases. The aetiology of IIM likely includes interactions between genetic and environmental factors. Several genetic variants common to multiple autoimmune disorders have been identified in recent genome-wide association studies (GWAS). A Myositis Genetics Consortium dermatomyositis (DM) GWAS also suggests genetic overlap with other autoimmune disorders.[1] We sought to

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extend these findings to identify novel genetic risk factors in a large cohort of adult/juvenile patients with DM and polymyositis (PM), by genotyping immune-related single nucleotide polymorphisms (SNPs) not captured through the DM GWAS.[1]

SNPs significantly associated ($p<5\times10^{-8}$) with 10 autoimmune disorders (systemic lupus erythematosus, rheumatoid arthritis, juvenile idiopathic arthritis, coeliac disease, Crohn's disease, ulcerative colitis, psoriasis, type 1 diabetes, multiple sclerosis and systemic sclerosis) were identified from published GWAS or the National Human Genome Research Institute GWAS catalogue.[2] Unique SNPs were identified (n=233), of which 99 had not been directly genotyped or captured ($r^2\geq0.8$ with genotyped SNPs) through our DM GWAS.[1] These 99 SNPs were genotyped using Sequenom in 1001 European Caucasian individuals with definite/probable adult or juvenile DM or PM.[34] Eighty-three SNPs passed quality control. GWAS data[2] were imputed to the 1000G_phase 1 integrated_v3 reference panel using IMPUTE2.[5] Concordance rates were >98% for 444/1001 cases overlapping the GWAS data set. INFO scores for the 83 SNPs were >0.6. The 1001 Sequenom cases were merged with 168 unique cases from the GWAS. Samples with >5% missing data were excluded, resulting in 1149 cases and 3572 controls (table 1). Association tests were performed for the 83 SNPs in DM and PM subgroups including juvenile cases, and the overall IIM group (SNPTEST V.2.4.1). Imputed genotype uncertainty was accounted for using the 'score' method. A random-effects meta-analysis of the individual country data sets was performed (META V.1.5). Local research ethics committee approval and informed consent were obtained.

Outside the major histocompatibility complex (MHC), a non-synonymous SNP rs2304256 in *TYK2* was identified reaching Bonferroni corrected significance in DM and the overall IIM group, but not in the PM subgroup (p=0.17) (table 2). *TYK2* encodes a member of the Janus tyrosine kinase protein family, contributing to cytokine receptor signaling via its catalytic activity or kinase-independent scaffolding function, and may play a role in antiviral immunity. The associated SNP is predicted to be damaging to protein function using SIFT (Sorting Intolerant From Tolerant, a program that predicts whether an amino acid substitution affects protein function), but benign by Polyphen. rs2304256 is in the protein FERM (4.1 protein, ezrin, radixin, moesin) domain, which mediates interaction with Janus kinase and microtubule interacting protein 1, suggesting a possible increased interaction in DM cases. *TYK2* has been associated with rheumatoid arthritis,[6] juvenile idiopathic arthritis,[7] systemic lupus erythematosus,[8] type 1 diabetes[9] and multiple sclerosis.[10] Two SNPs 2335 bp 5′ of *BLK* (rs13277113) and within *BLK* (rs2618476) were associated with DM but not PM (table 2). These SNPs are highly correlated ($r^2>0.95$, HapMap CEU) with rs2736340, 7.5 kb 5′ of *BLK*, supporting the previous DM GWAS results,[1] and suggesting a role of B cells in development of DM. No non-MHC SNPs were associated with PM at a Bonferroni corrected significance level.

We have identified *TYK2* as a novel associated locus for DM. This study confirms *TYK2* as an autoimmune gene and suggests a genetic overlap of DM with other autoimmune disorders, indicating genetic heterogeneity between PM and DM, although the difference may be due to the smaller PM sample size. These results require replication in an independent cohort and functional studies of the pathogenic role of *TYK2* in myositis. Our

analysis supports the study of additional immune-related loci in larger cohorts, using SNP arrays such as the Immunochip.

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Table 1

Polymyositis, dermatomyositis and matched control sample numbers included in the final analysis

	DM cases			PM cases				
Country	**JDM**	**DM**	**DM total**	**JPM**	**PM**	**PM total**	**Cases total**	**Controls total**
1. Czech Republic	11	134	145	–	61	61	206	166
2. Hungary	14	71	85	2	79	81	166	90
3. Netherlands/Sweden	4	54	58	–	67	67	125	642
4. Spain	4	61	65	–	–	–	65	259
5. UK	183	203	386	6	195	201	587	2415
TOTAL	216	523	739	8	402	410	1149	3572

DM, dermatomyositis; JDM, juvenile dermatomyositis; JPM, juvenile polymyositis; PM, polymyositis.

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Table 2

SNPs associated with dermatomyositis, polymyositis or IIM

Phenotype	Chr	SNP	Position (hg19)	Locus*	p Value	Risk/non-risk allele	RAF cases	RAF controls	β	p Heterogeneity	Disease association for non-HLA SNPs[3]
DM	**6**	**rs116152465**	**32603007**	**5′ HLA-DQA1**	**1.48542E-05**	**G/A**	**0.25667**	**0.20135**	**0.40**	**0.31**	
DM	**19**	**rs2304256**	**10475652**	**TYK2**	**0.00020**	**C/A**	**0.75478**	**0.7067**	**−0.25**	**0.59**	**T1DM**
DM	**6**	**rs114424451**	**32413051**	**3′ HLA-DRA1**	**0.00021**	**G/A**	**0.91272**	**0.86887**	**0.34**	**0.99**	
DM	**8**	**rs13277113**	**11349186**	**5′ BLK**	**0.00045**	**A/G**	**0.28098**	**0.24403**	**0.25**	**0.54**	**SLE**
DM	**8**	**rs2618476**	**11352541**	**BLK**	**0.00062**	**C/T**	**0.28903**	**0.25312**	**0.24**	**0.56**	**SLE**
DM	9	rs7020673	4291747	GLIS3	0.0023	C/G	0.53583	0.49292	−0.18	0.64	T1DM
DM	5	rs6859219	55438580	ANKRD55	0.0023	C/A	0.82304	0.79178	−0.24	0.80	RA
DM	2	rs13385731	33701890	RASGRP3	0.0049	T/C	0.947972	0.928019	−0.38	0.67	SLE
DM	12	rs11171739	56470625	5′ ERBB3	0.0069	T/C	0.62421	0.57102	0.28	0.09	T1DM
DM	3	rs11712165	119118796	ARHGAP31	0.0071	G/T	0.40245	0.38066	0.16	0.85	Celiac
DM	2	rs10210302	234158839	5′ ATG16L1	0.0094	C/T	0.51259	0.46989	−0.17	0.36	Crohn’s
PM	**6**	**rs116152465**	**32603007**	**5′ HLA-DQA1**	**0.00044**	**G/A**	**0.33252**	**0.20135**	**0.76**	**0.01**	
PM	6	rs114424451	32413051	3′ HLA-DRA1	0.0033	G/A	0.910976	0.86887	0.35	0.53	
PM	6	rs615672	32574171	Intergenic (HLA-DRB1)	0.0035	C/G	0.51585	0.35732	0.52	0.01	
PM	14	rs4900384	98498951	Intergenic (c14orf64)	0.0072	A/G	0.67073	0.71415	0.23	0.74	T1DM
IIM	**6**	**rs114424451**	**32413051**	**3′ HLA-DRA1**	**9.09513E-06**	**G/A**	**0.912097**	**0.86887**	**0.35**	**0.88**	
IIM	**6**	**rs116152465**	**32603007**	**5′ HLA-DQA1**	**1.04525E-05**	**G/A**	**0.28442**	**0.20135**	**0.51**	**0.06**	
IIM	**19**	**rs2304256**	**10475652**	**TYK2**	**0.00027**	**C/A**	**0.74672**	**0.7067**	**−0.21**	**0.51**	**T1DM**
IIM	12	rs11171739	56470625	5′ ERBB3	0.0054	T/C	0.62753	0.57102	0.30	0.02	T1DM
IIM	11	rs6590330	128311059	Intergenic (ETS1)	0.0064	A/G	0.12931	0.10279	0.22	0.65	SLE
IIM	17	rs7221109	38770286	Intergenic (SMARCE1)	0.0071	T/C	0.37882	0.34209	−0.15	0.44	T1DM
IIM	2	rs10210302	234158839	5′ ATG16L1	0.0071	C/T	0.50503	0.46989	−0.14	0.45	Crohn’s
IIM	8	rs13277113	11349186	5′ BLK	0.0077	A/G	0.27101	0.24403	0.21	0.24	SLE
IIM	3	rs11712165	119118796	ARHGAP31	0.0080	G/T	0.39723	0.38066	0.14	0.80	Celiac
IIM	8	rs2618476	11352541	BLK	0.0082	C/T	0.279	0.25312	0.20	0.27	SLE

SNPs in bold reached Bonferroni significance.

*Gene is annotated where closest gene is <10 kb, otherwise annotated as intergenic with closest gene in parentheses. Chr, chromosome; DM, dermatomyositis; HLA, human leukocyte antigen; IIM, idiopathic inflammatory myopathy; PM, polymyositis; p value, p value from random-effects meta-analysis; p heterogeneity, p value of test for heterogeneity; β, effect size (META V.1.5); RA, rheumatoid arthritis; RAF, risk allele frequency; SLE, systemic lupus erythematosus; SNP, single nucleotide polymorphism; T1DM, type 1 diabetes mellitus.